Short title:

Phosphorus, TB and HIV

Full title:

HIV, TB, inflammation and other correlates of serum phosphate:

a cross-sectional study

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ABSTRACT

Background: There is little information about serum phosphate levels among patients with pulmonary tuberculosis (TB) and HIV infection.

Objective: We aimed to ~~to~~ assess the role of TB, HIV, inflammation and other correlates ~~of~~ on serum phosphate levels.

Methods: A cross-sectional study was conducted among TB patients and age- and sex-matched non-TB controls. Pulmonary TB patients were categorized as sputum-negative ~~(TB-)~~ and -positive ~~(TB+)~~, based on culture. Age- and sex-matched non-TB controls were randomly selected among neighbors to ~~TB+~~ sputum-positive TB patients. Data on age, sex, alcohol and smoking habits were obtained. HIV status, serum phosphate, and the acute phase reactants C-reactive protein (serum CRP) and α_1-acid glycoprotein (serum AGP) were determined. Linear regression analysis was used to identify correlates of serum phosphate.

Results: Of 1605 participants, 355 (22.1%) were controls and 1250 (77.9%) TB patients, of which 9.9% and 50.4% were HIV-infected. Serum phosphate was determined before start of TB treatment in 44%, and 1-14 days after start of treatment in 56%. Serum phosphate was up to 0.10 mmol/L higher 1-3 days after start of TB treatment, and lowest 4 days after treatment, after which it increased. In multivariable analysis, TB patients had 0.09 (95%CI: 0.05; 0.13) mmol/L higher serum phosphate than controls, and those with HIV had 0.05 (95%CI: 0.01; 0.08) mmol/L higher levels than those without. Smoking was also a positive correlate of serum phosphate, whereas male sex and age were negative correlates.

Conclusion: While HIV and TB are associated with higher serum phosphate, our data suggest that TB treatment is followed by transient reductions in serum phosphate, which may reflect hypophosphataemia in some patients.

Key words: Serum phosphate, phosphorus, tuberculosis; HIV, acute phase response

INTRODUCTION

Phosphorus is an essential mineral in the body, being ~~,~~ a structural component of DNA and RNA and of membranes, and important for metabolism and storage of energy (1). Phosphorus is likely to be a limiting nutrient among individuals in low-income settings, since the typical diet is low in animal-source foods and high in cereals (2). Although cereals, including maize, have a high content of phosphorus in the form of phytic acid (~~, ie~~ inositol hexaphosphate)~~,~~ it is largely unabsorbable (2,3).

Data on phosphorus status ~~are~~ is scarce, even among low-income individuals where phosphorus deficiency is likely to be common. Although serum phosphate is known to be a poor marker of phosphorus status, it is the only available (3), and low values are related to various clinical conditions. For example, serum phosphate is measured to monitor patients at risk of refeeding hypophosphataemia, a potentially fatal condition that arises when high energy feeding is initiated in ~~as~~ phosphorus depleted individuals ~~suddenly are fed high amounts of energy~~ (4). As refeeding restores metabolism, it also leads to further intracellular phosphorus depletion, due to utilization of phosphorus for anabolic processes and storage of energy through phosphorylation of ADP to ATP~~. Such refeeding hypophosphatemia potentially results in multi-organ failure and death~~ (5).

Recently, there has been a renewed interest in serum phosphate in patients with HIV infection on antiretroviral treatment (ART). Tenofovir has been shown to cause renal tubular function abnormalities (6), which is a known cause of hypophosphataemia (7,8). Among HIV patients starting ART in Zambia, a case of acute hypophosphataemia was described (9), and low serum phosphate prior to ART was found to be a predictor of early mortality among those with low BMI (10,11). Fortification of a lipid-based nutritional supplement with vitamins and minerals reduced renal wasting of phosphate among malnourished Zambian patients starting ART (2).

As part of a larger nutrition study, we obtained cross-sectional data on serum phosphate among pulmonary TB patients and age- and sex-matched neighbourhood controls, with an aim to assess the level of serum phosphate and the role of pulmonary TB, HIV, the acute phase response and other potential correlates.

METHODOLOGY

Ethics Statement

Ethical permission was obtained from the Medical Research Coordinating committee of the National Institute for Medical Research in Tanzania, and consultative approval was given by The Danish Central Medical Ethics Committee. Written and oral information was presented to all eligible participants by the health staff before written informed consent was obtained. Written consent was obtained from parents/legal guardians of any participant under 18 years of age.

Study setting and design

A cross-sectional study was conducted from April 2006 to March 2009 in Mwanza City, Tanzania, among TB patients recruited for a large nutrition intervention study and non-TB controls. Mwanza City is at the shores of Lake Victoria. The harvest is from May to July, and the staple foods are maize, cassava, sweet potato, rice, and millet. Fish is the most common animal-source food. ~~and s~~Small fish are often eaten whole, ~~.~~ but only 25% eat them >4 days per week (12).

Recruitment and management of TB patients

The TB patients were recruited at the four TB clinics under the TB treatment services, coordinated by the National Tuberculosis and Leprosy Programme. If residents of Mwanza city, ~~Both~~ both ~~smear~~sputum-positive ~~(TB+)~~ and ~~smear~~sputum–negative ~~(TB-)~~ TB patients, based on culture, were enrolled in the study after giving informed consent ~~if they were residents of Mwanza city~~. Patients were excluded if pregnant, under the age of 15 years or were suffering from ~~with~~ extra-pulmonary TB ~~, pregnancy, age under 15 years,~~ or a terminal illness ~~were excluded~~. The diagnosis of TB followed the World Health Organization (WHO) guidelines (13) using the Ziehl–Neelsen staining technique (14). Briefly, all patients suspected of having TB were asked to bring three sputum samples for microscopy, and chest X-rays were done as appropriate. Patients were considered to be ~~smear~~sputum-positive, if two samples tested positive or one sample tested positive and a chest X-ray was suggestive of TB, and to be ~~smear~~sputum-negative TB patients if all the samples were negative, but chest X-ray and clinical suspicion was suggestive of TB, and there was non-response to a course of broad-spectrum antibiotics. After diagnosis all patients were started on a standardized TB treatment for 6-8 months based on existing national guidelines (15,16).

Those found to be HIV-infected were referred for management based on national guidelines at the time of the study (17). ~~At~~ On the day TB treatment was started, the patients also started daily supplementation as part of two nutrition intervention trials. In the energy-protein trial, those found ~~TB+~~sputum-positive and HIV co-infected were randomized to receive one or six energy–protein biscuit bars daily (18). One of the biscuit bars given to the experimental group and the one given to the control group contained additional micronutrients, so that the micronutrient intake was similar in the two groups. All other TB patients were randomized to a daily biscuit bar with or without additional micronutrients (19). Each biscuit bar weighed 30 g and contained 4·5 g protein, 615 kJ energy, and 120 mg P.

Recruitment of non-TB controls

~~400~~ Four hundred consecutive ~~smear~~sputum-positive participants were considered index cases for selection of age- and sex-matched neighbourhood non-TB controls. Mwanza City is divided into wards, streets and communal cells. Each cell has 10-20 households, and is headed by a ten-cell leader. Each of the index patients was asked to provide his/her residential address and the name of his/her ten-cell leader. Using this information, the study team requested the ten-cell leader to provide the complete list of individuals in his/her jurisdiction meeting the age and sex recruitment criteria. Of these, one was randomly selected using a lottery method and invited to participate in the study as a non-TB control if meeting the following criteria: no history of previous TB exposure, active TB or TB treatment, no evidence of current active TB (cough, intermittent fevers, and excessive night sweating in the past two weeks and unexplained weight loss in the past month), same sex as index case, aged 15 years or above and age difference from index case less than five years, had lived in the same street as index case for at least three months, not pregnant, and consenting to participate in the study. Persons who were terminally ill were not invited. The recruitment of non-TB controls was done in parallel with inclusion of cases from October 2006 to January 2009.

Data collection

For the purpose of the study, all TB patients provided an additional sputum sample for culture at the Zonal TB Reference Laboratory, and were subsequently categorized as ~~TB~~sputum~~+~~-positive or ~~TB~~sputum-negative, based on culture. For missing or contaminated culture samples, the initial evaluation from sputum smear microscopy was used. All TB patients and controls had data on demography, smoking, and alcohol intake collected using questionnaires, while data on ART ~~were~~ was retrieved from ART-use databases in ART clinics. Morning venous blood was collected in a 10 ml plain vacutainer tube for HIV testing and a 5 ml EDTA vacutainer tube for CD4 count. This was preferably done immediately prior to start of TB treatment, but could for logistical reasons be delayed; median delay was ~~. As previously reported, the median (range) delay of blood sampling was~~ 1 (range 0–14) days after initiation of TB treatment (20). All tubes were cooled on dry ice before transported to the laboratory, where they were centrifuged and serum samples ~~were~~ stored at -80°C. HIV status was determined using Capillus HIV-1/HIV-2 (Trinity Biotech Plc., Wicklow, Ireland) and Determine HIV-1/HIV-2 (Inverness Medical Innovations, Inc., Delaware, U.S.A.) tests in parallel. HIV infection was diagnosed if both tests gave a positive result and HIV negative diagnosis was made if both tests produced a negative result. Indeterminate results were resolved using ELISA– Organon Uniform II (Organon Teknia Ltd, Boxtel, Netherlands). CD4 count was determined as cells/µl using a Partec Cyflow Counter (Partec GmbH, Münster**,** Germany). The biochemical analyses, ie acute phase reactants and phosphate, were conducted at Aalborg University Hospital, FBE Clinical Biochemistry South. Serum phosphate was determined using Phosphate (Inorganic) ver.2 (PHOS2) on a Cobas 6000 instrument from Roche (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The cut-offs used to define low and high serum phosphate were 0.80 and 1.60 mmol/L (3). Serum α_1-acid glycoprotein (AGP) was determined with a standard Alpha1-Acid Glycoprotein Kit using Beckman Coulter Image*H* Immunochemistry Systems (Beckman Coulter, Galway, Ireland) and C-reactive protein (CRP) was determined with Tina-quant C-Reactive Protein Gen.3 (CRPL3) on a Roche COBAS 6000 instrument (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Normal probability plots were used to assess the distribution of continuous variables. Chi-square test was used to test for differences in proportions. To assess and adjust for the effect of TB treatment and nutritional interventions on serum phosphate in case blood sampling was delayed, a variable was created to express the number of days delay after start of TB treatment. For controls and those treated the same day or the day after blood sampling this variable was given the value 0. The two-sample t test or oneway ANOVA were used to test for differences in means between two or more groups, respectively, and Scheffe post hoc tests were used to adjust for multiple comparisons. Linear regression analysis was used to identify correlates of serum phosphate. The variables assessed were age, sex, smoking, consumption of alcohol, TB and HIV status, and serum CRP or AGP. Age and sex, and variables found significant in the univariate analyses were assessed in a final multivariable analysis, with all variables included, with and without adjustment for elevated levels of either serum CRP or AGP. Year and month of recruitment and delay in blood sampling since initiation of TB treatment were adjusted for. We examined normal and residual-vs.-fitted plots to assess normality and homoscedasticity of residuals. Stata version 12.1 (StataCorp, Texas, USA) was used for all analyses.

Results

Of the 1605 study participants, 355 (22.1%) were controls and 1250 (77.9%) TB patients. Culture data were available on 1142 (91.4%) of the 1250 TB patients. In the remaining 108 (8.6%) cultures were contaminated or missing, and the categorization of TB patients as sputum-negative and sputum-positive therefore based on microscopy. Thus, of the 1250 TB patients~~, of which~~ 427 (34.2%) were ~~TB~~sputum-negative and 823 (65.8%)~~ TB+.~~ sputum-positive (Table 1). As previously reported (19), the HIV prevalence was higher among TB patients compared to controls (50.4 vs 9.9%, p<0.001), and higher among sputum-negative~~TB-~~ compared to ~~TB+~~sputum-positive patients (64.4 vs 43.1%, p<0.001). The mean BMI was 18.8 among TB patients and 22.6 among controls (p<0.001). Data on serum phosphate were available for 1522 (94.8%) of the 1605 participants. Among 349 controls, mean (±SD) serum phosphate was 1.14 (±0.28) mmol/L with 4.3% (n=15) having values below 0.80 mmol/L, and 4.9% (n=17) above 1.6 mmol/L, respectively. Among 1173 TB patients the mean (±SD) serum phosphate was 1.27 (±0.29) mmol/L, and 2.2% (n=26) had values below 0.80 mmol/L, and 9.2% (n=108) above 1.6 mmol/L. Of these, 518 (44%) had blood samples taken before start of TB treatment, while 218 (18.6%) were bled with 1 day delay, and the remaining with 2-14 days delay. Those bled with delay were 2.3 (95% CI: 0.8; 3.7) years older, had a higher prevalence of HIV (54.7 vs 45.5%, p=0.01), whereas there was no difference in sex distribution (p>0.30). As seen in ~~the~~ **Figure 1**, unadjusted mean serum phosphate was up to 0.10 mmol/L higher in those bled 1-3 days after start of TB treatment, and lowest in those bled with 4 days delay, after which it seemed to increase with number of days delay. Numbers were too small to allow stratification by nutritional intervention.

Mean serum phosphate by category of sex, age, smoking, alcohol consumption, pulmonary TB and HIV is shown in **Table ~~1~~2**, with TB patients and controls combined. There were no differences by sex and age in univariate analyses, but serum phosphate were higher in those smoking or taking alcohol. There was no difference in serum phosphate between ~~TB-~~sputum-negative and ~~TB+~~sputum-positive TB patients (1.26 vs 1.27 mmol/L, p=0.41). However, TB patients together had higher serum phosphate than controls (1.27 vs 1.14 mmol/L, p<0.0001). The difference was similar if tested only among the index cases and controls (1.26 vs 1.14 mmol/L, p<0.0001; not shown in table). HIV+ patients had higher serum phosphate than HIV- (1.29 vs 1.21 mmol/L, p<0.0001), irrespective of ART status. The association between HIV status and serum phosphate was not different between TB patients and controls (interaction, p=0.56, data not shown). While serum phosphate was higher among those with HIV, it was lower in those with CD4 counts below 250 compared to above 500 cells/µL, although the difference was only marginally significant (p=0.08, Scheffe post-hoc). Elevated serum CRP or AGP were both associated with higher serum phosphate.

The results of a multivariable analysis, with adjustment for year and months of recruitment and delay in blood sampling, are shown in **Table ~~2~~3**. The relationship between age, sex, HIV, smoking and serum phosphate were not different between TB patients and controls (interaction, p>0.10). Without adjustment for elevated serum AGP (**model 1**), serum phosphate was lower in males compared to females, and lower in those above 25 years of age. Alcohol intake was not associated with serum phosphate, but current smoking was associated with higher levels. TB patients had 0.09 (95% CI: 0.05; 0.13) mmol/L higher serum phosphate compared to the non-TB controls, whereas there was no difference between sputum-positive~~TB+~~ and ~~TB-~~sputum-negative patients. Finally, those with HIV infection had 0.05 (95% CI: 0.01; 0.08) mmol/L higher levels than those without. The associations with delayed bleeding, assessed in this multivariable model, was similar to what was shown in ~~the~~ Figure 1. As such, delays for 1 to 3 days were associated with 0.10 (95% CI: 0.06; 0.15), 0.01 (95% CI: -0.06; 0.09) and 0.08 (95% CI: 0.02; 014) higher serum phosphate, while delay to day 4 was associated with 0.06 (95% CI: -0.01; 0.13) lower serum phosphate. If days since TB treatment were not adjusted for, then the regression coefficient for TB was 0.12 (95% CI: 0.08; 0.15).

Elevated serum AGP was a strong positive correlate of serum phosphate, while elevated serum CRP was not, in multivariable analysis. Adjustment for elevated serum AGP (Table ~~2~~3, **model 2**) considerably reduced the regression coefficient of ~~TB+~~sputum-positive TB (from 0.09 to 0.03 mmol/L), whereas that of HIV and other correlates did not change considerably. Compared to the overall mean serum phosphate of 1.24 (95% CI: 1.23; 1.25), the intercept was 1.20 (95% CI: 1.13; 1.26), and reflects the mean among individuals in all reference categories, ie young, non-smoking females without TB, HIV and elevated serum AGP. While no interaction between age and sex was found (p=0.40), there was an interaction between age and sex among controls (p=0.01). The interaction reflected a decline in serum phosphate per 10 year increase in age among males (-0.04, 95%CI: -0.08; -0.010, p=0.01), but not among females (0.01, 95%CI: -0.03; .05, p=0.56).

Discussion

Hypophosphataemia

We found that serum phosphate, compared to those examined before TB treatment start, was higher in those examined 1-3 days after ~~start of TB treatment~~, but lower in those examined 4 days after. While selection bias cannot be excluded, this pattern more likely reflects changes in phosphate metabolism due to the commencement of TB treatment with regain in appetite, and increased food intake, from the diet as well as from the supplements provided as part of the trials. The nadir at day 4 may reflect that some TB patients could have refeeding hypophosphataemia. In the classical description of refeeding syndrome, starved individuals refed with high amounts of glucose and amino acids developed hypophosphataemia accompanied by cardio-pulmonary failure. The existence of a similar syndrome among HIV patients starting ART has been suggested (9–11), whereas it does not seem to have been studied among TB patients. Nevertheless, the risk and magnitude of refeeding hypophosphataemia after initiation of TB-treatment may depend on the initial phosphorus status, as well as the intake of energy and bioavailable phosphorus and other bulk minerals and probably vitamins. There is currently increasing awareness that patients with TB need nutritional care and support (21), and it is important to ensure adequate intake of phosphorus ~~to~~ not only to prevent refeeding syndrome, but also to support regain in lean mass and body functions.

After adjustment for the effect of delayed blood sampling, TB was associated with 0.09 mmol/L higher serum phosphate, compared to no TB, much of which was explained by elevated serum acute phase reactants. Yet, there are several reasons to believe that phosphorus status was low. First, the staple food is maize and the intake of animal source foods is limited. ~~This is supported by a relatively low mean serum phosphate among controls (1.14 mmol/L), although within the reference interval was 0.80 and 1.60 mmol/L, with 4.3% having low values~~. Second, the TB patients in the study had typically been ill for some time, and we have previously shown that they have an average weight deficit of 9 kg (22). The weight deficit is due to lower habitual weight as well as reduced food intake and increased utilization of energy as a result of the TB disease itself. Since inflammation-induced wasting to a large extent is due to catabolism of lean mass, this may result in increased serum phosphate. It has been shown in children with inflammatory bowel disease, another condition involving systemic inflammation, that flare-up leads to a sustained upregulation (reduced degradation) of the phosphatonin Fibroblast Growth

Factor 23 (FBG23), which increases renal phosphorus excretion (23). Hence, although speculative, it is likely that patients with TB, despite the elevated serum phosphate, are phosphorus depleted, and may continue to have high urinary phosphorus excretion for some time. This will contribute to increase the phosphorus requirements during the critical period of convalescence when there is a need to rebuild lean mass, ie organ and muscle.

HIV infection

We found 0.05 mmol/L higher serum phosphate in those with compared to without HIV infection, both among TB patients and controls. Among HIV patients, serum phosphate was not different in the 76 ART-treated compared to the 558 ART-naïve patients. The main ART regimen used was stavudine/lamivudine/nevirapine, whereas tenofovir, known to cause hypophosphataemia as part of Fanconi's syndrome (6,7), was not used. In contrast to TB, only a minor part of the association between HIV and serum phosphate was explained by the acute phase response. In a trial among malnourished Zambian and Tanzanian HIV patients starting ART, there were complex interrelationships between serum phosphate and early mortality which were accentuated by vitamin and mineral fortification of a lipid-based supplement (24,25)~~(Woodd et al., submitted for publication; Rehman et al., submitted for publication)~~ although the supplement improved renal phosphate retention (2). The results suggested it was variability of serum phosphate, possibly due to poor metabolic control among malnourished, seriously ill patients, which was associated with mortality risk (25) ~~(Rehman et al., submitted for publication)~~.

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Serum phosphate was lower in males compared to females and in the higher compared to lower age groups in the multivariable models. However, among controls only, we found an interaction between age and sex, due to a decline in serum phosphate with increasing age in males, but not in females. The overall decline of serum phosphate with age has been reported from several population based studies (26,27). A large ~~US~~ study from the USA found a decline with age in men. However, in women, there was ~~that the lack of a consistent~~ a decline ~~in serum phosphate with age among females concealed a decline~~ up to 44 years ~~as for males~~, and then a transient increase with the onset of menopause, in parallel with changes in tubular phosphate reabsorption (28). While this age-sex pattern was also seen among healthy adults in Tanzanians, it disappeared among TB patients, even after adjustment for other factors. The higher serum phosphate in smokers is also in accordance with previous studies (29), and may be explained by greater bone loss in smokers.

Despite the limitations of serum phosphate as a marker of phosphorus status, and of our cross-sectional design to draw conclusions about cause-effect relationship, the study suggests that some patients may experience refeeding hypophosphataemia a few days after start of treatment.

Table 1. Diagnosis of 1250 tuberculosis patients as sputum-negative or sputum-positive

	Sputum status [1]		
	Positive [2]	**Negative [3]**	**Total (%)**
Culture	754 [4]	388	1142 (91,4)
Microscopy	69	39	108 (8,6)
Total (%)	823 (65.8)	427 (34,2)	1250 (100%)

[1] Sputum status was based on culture, if available, otherwise microscopy.
[2] Patients were considered to have sputum-positive tuberculosis, if two samples tested positive or one sample tested positive and a chest X-ray was suggestive of tuberculosis.
[3] Patients were considered to have sputum-negative tuberculosis if all the samples were negative, but chest X-ray and clinical suspicion was suggestive of tuberculosis, and there was non-response to a course of broad-spectrum antibiotics.
[4] Based on 400 consecutive sputum-positive index cases 400 age- and sex-matched neighbourhood non-TB controls were selected

Table 2. Serum phosphate (mmol/L) among 1173 of 1250 pulmonary TB patients and 349 of 355 non-TB neighbourhood controls by categories of sex, age, smoking, pulmonary TB, HIV and serum acute phase reactants [1]

	% (n)	Mean (SD)	95% CI	P
Sex				
Females	42.0 (639)	1.25 (0.28)	1.23; 1.28	0.11
Males	58.0 (883)	1.23 (0.31)	1.21; 1.25	
Age (y)				
<25	21.9 (333)	1.27 (0.28)	1.23; 1.30	0.16
25-45	58.2 (886)	1.24 (0.30)	1.22; 1.25	
45+	19.9 (303)	1.23 (0.31)	1.19; 1.26	
Smoking				
Never	71.3 (1073)	1.23 (0.30)	1.21; 1.25	0.04
Previously	8.9 (134)	1.27 (0.27)	1.22; 1.31	
Currently	19.8 (297)	1.27 (0.28)	1.24; 1.30	
Alcohol intake				
No	58.0 (883)	1.23 (0.29)	1.21; 1.24	0.03
Yes	42.0 (638)	1.26 (0.31)		
Pulmonary TB status [1]				
Non-TB control	22.9 (349)	1.14 (0.28)	1.11; 1.17	<0.0001
~~TB-~~Sputum-negative TB	26.7 (406)	1.26 (0.27)	1.23; 1.29	
~~TB+~~Sputum-positive TB	50.4 (767)	1.27 (0.31)	1.25; 1.30	
HIV and ART status				
HIV-	58.3 (888)	1.21 (0.26)	1.19; 1.22	<0.0001
HIV+ not on ART	36.7 (558)	1.29 (0.34)	1.26; 1.31	
HIV+ on ART	5.0 (76)	1.28 (0.29)	1.22; 1.35	
CD4 count (cells/μL)				
HIV-	58.4 (888)	1.21 (0.26)		<0.0001
500+	6.4 (97)	1.35 (0.32)	1.28; 1.41	
250-500	12.6 (191)	1.30 (0.31)	1.26; 1.34	
<250	22.7 (345)	1.26 (0.35)	1.22; 1.30	
Serum C-Reactive Protein (mg/L)				
≤ 2	20.1 (305)	1.16 (0.28)	1.13; 1.20	<0.0001
2-10	13.3 (201)	1.19 (0.24)	1.15; 1.22	
10-50	18.2 (275)	1.24 (0.26)	1.21; 1.27	
50-100	25.7 (390)	1.26 (0.27)	1.23; 1.28	
100+	22.7 (344)	1.32 (0.36)	1.28; 1.35	
Serum α_1-Acid Glycoprotein (mg/L)				
≤ 1	25.6 (389)	1.15 (0.27)	1.13; 1.18	<0.0001
1-2	20.1 (305)	1.22 (0.27)	1.19; 1.25	
2-3	37.4 (567)	1.26 (0.26)	1.24; 1.28	
3+	16.9 (257)	1.35 (0.39)	1.30; 1.40	

[1] Pulmonary TB status was based on culture, except where culture data were not available. For 355 consecutively recruited sputum-positive TB patients a control was randomly selected among individuals

from the neighbourhood with same sex and age. Serum phosphate data were available on 1522, but n may sum up to less, due to missing data on smoking (n=18), alcohol intake (n=1), CD4 count (n=1), serum C-Reactive Protein (n=7), serum α1-Acid Glycoprotein (n=4). P-values were based on t-test and oneway. TB is tuberculosis, HIV is human immunodeficiency syndrome, ART is antiretroviral treatment

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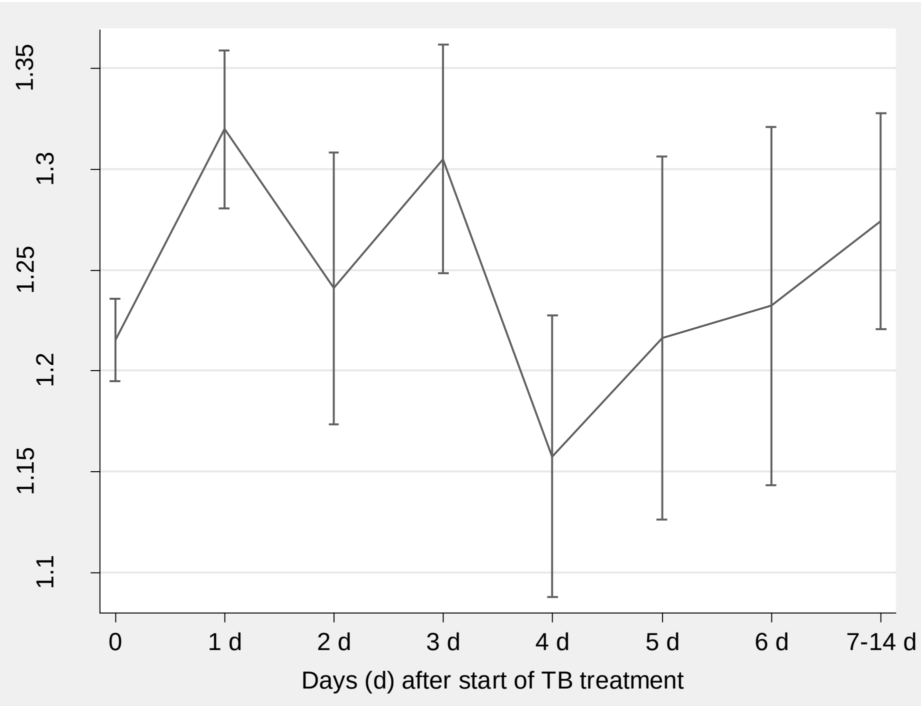
Table 3. Multivariable models with correlates of serum phosphate ~~in~~ among 1173 of 1250 pulmonary TB patients and 349 of 355 non-TB neighbourhood controls with regression coefficient B, 95% confidence interval (CI) and P-values [1]

	Model 1 [2]			Model 2 [3]		
	B	95% CI	P	B	95% CI	P
Sex						
Female	-					
Male	-0.04	-0.07; -0.005	0.02	-0.04	-0.07; -0.01	0.02
Age (years)						
<25	-					
25-45	-0.06	-0.10; -0.03	0.001	-0.06	-0.10; -0.02	0.001
45+	-0.07	-0.12; -0.02	0.003	-0.06	-0.10; -0.01	0.02
Smoking						
Never	-					
Previously	0.04	-0.01; 0.10	0.12	0.04	-0.01; 0.10	0.13
Currently	0.06	0.02; 0.10	0.004	0.06	0.02; 0.10	0.004
TB status [1]						
Non-TB control	-			-		
TB	0.09	0.05; 0.13	<0.0001	0.03	-0.03; 0.09	0.34
HIV status						
HIV-	-					
HIV+	0.05	0.01; 0.08	0.004	0.04	0.01; 0.07	0.02
Serum α_1-acid glycoprotein (mg/L)						
<1				-		
1-2				0.02	-0.04;0.08	0.56
2-3				0.05	-0.01; 11	0.10
3+				0.14	0.07; 0.20	<0.001

[1] Pulmonary TB status was based on culture, and microscopy only if culture data were not available. For 355 consecutively recruited sputum positive TB patients a control was randomly selected among individuals with same sex and age from the neighbourhood. [2] Model 1: N=1491, adjusted R^2=0.07 and intercept=1.20 (95% CI: 1.14; 1.27). [3] Model 2: N=1487, adjusted R^2=0.09 and intercept=1.20 (95% CI: 1.13; 1.26). Both models contained all the variables and were adjusted for year and months of recruitment and days since start of TB treatment. TB is tuberculosis, HIV is human immunodeficiency syndrome

Figure 1. Serum phosphate by day after start of of TB treatment. Based on linear regression, with adjustment for TB, and non-TB controls and TB patients commencing TB treatment before or at the day of blood sampling coded as 0. Number of participants: day 0 (n=867), 1 (n=218), 2 (n=72), 3 (n=102), 4 (n=67), 5 (n=40), 6 (n=41) and 7-14 (n=115).



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